Influence of bioresorbable, unsintered hydroxyapatite/poly-L-lactide composite films on spinal cord, nerve roots, and epidural space

Masanori Matsumoto,[1] Etsuo Chosa,[1] Kazuki Nabeshima,[2] Yasuo Shikinami,[3] Naoya Tajima[1]
[1]*Department of Orthopedics, Miyazaki Medical College, 5200, Kiwara, Kiyotake-cho, Miyazaki 889-1692, Japan*
[2]*Department of Pathology, Miyazaki Medical College, 5200, Kiwara, Kiyotake-cho, Miyazaki 889-1692, Japan*
[3]*Takiron Co., Ltd., Medical Division, 405, Nagano, Yasutomi-Cho, Hyogo 671-2421, Japan*

Received 19 December 2000; revised 12 June 2001; accepted 19 June 2001
Published online 17 January 2002

Abstract: The effect of forged unsintered hydroxyapatite/poly-L-lactide (u-HA/PLLA) composite films on spinal cord and nerve roots and its degradation behavior and osteoconductivity in epidural space were compared with those of calcined HA (c-HA)/PLLA and unfilled PLLA films. Partial laminectomy was performed on 20 rabbits, and u-HA/PLLA and PLLA films were implanted in the intervertebral space. Total laminectomy was performed on 30 rabbits to implant u-HA/PLLA, c-HA/PLLA, and PLLA films in both epidural and subcutaneous spaces. For up to 50 weeks, there were no histological changes in the spinal cord or nerve root, and no inflammatory cell infiltration into the epidural space around the films. The rate of decrease in viscosity average molecular weight of both composite films was initially higher than that of PLLA but eventually became lower, although there was no difference in the degradation behavior of the three films in either the epidural or subcutaneous spaces after 50 weeks. Scanning electron microscopic and energy-dispersive X-ray analysis indicated calcium phosphate deposits on the surface of composite films with new bone formation from 4 weeks. The u-HA/PLLA composite film therefore has good biocompatibility, osteoconductivity, and fast primary degradation rate, which may prove compatible with application to spinal surgery. © 2002 John Wiley & Sons, Inc. J Biomed Mater Res 60: 101–109, 2002

Key words: poly-L-lactide; hydroxyapatite; composites; spinal cord; bioactivity

INTRODUCTION

Most spinal prosthetics are made of stainless steel or titanium. However, these can corrode over extended periods. They can also cause cancer,[1] bone resorption, or in some cases, inflammation[2] due to micromotion or stress shielding as well as loosening. When metallic devices are placed in inaccessible locations, repair or dislodgement of damaged and rotated devices is difficult.[3] Metallic prosthetics interfere with diagnosis of postsurgical paralysis by magnetic resonance imaging (MRI) or computerized tomography (CT).

Bioresorbable bone fixation devices constructed from materials such as poly-L-lactide (PLLA) were developed recently to solve these problems and satisfactory results have been reported.[4–6] Bioresorbable polymetric devices have the following advantages: removal is not necessary; there is no restriction of bone growth owing to the gradual decrease in mechanical strength; the risk of osteoporosis due to stress shielding is lower; there is no metallic corrosion to cause tissue reactions; and there is no artefact in CT or MRI.[7,8] Bioresorbable devices have been used for stabilization of transplanted bone and inducted vertebral arch and for facet fusion[9] in spinal surgery. Because of their effectiveness, it is predicted that bioresorbable devices will supplant the use of metallic devices. However, bioresorbable devices do have certain problems: complications such as noninflammatory swelling, fissuration, or synovitis have been reported; degradation and complete absorption of PLLA are too slow; and devices with osteoconductivity and bone-bonding capability should be made of other bioactive materials; also, they are radiolucent, which complicates intraoperative control and postoperative diagnosis.

Santavirta et al.[10] reported that noninfectious, transient effusions from rapid degradation were seen in ~5% of clinical cases, including a group in which polyglycolic acid (PGA) was used for bone fracture treatment. Böstman et al.[11–13] observed a marked increase in free degradable polymers that was beyond the phagocytic capability of macrophages because of

Correspondence to: M. Matsumoto; E-mail: tomo-m@athena.ocn.ne.jp

DOI 10.1002/jbm.1283

the rapid degradation process; foreign body reactions arose from increased osmotic pressure. Bergsma et al.[14,15] reported nonspecific foreign body reactions to PLLA bone plates and screws used for the fixation of zygomatic fractures 3 years after implantation. They concluded, without considering the high molecular weight and crystallinity of PLLA, that PLLA did not cause cellular reactions but that tissue reactions occur from very slow hydrolysis of PLLA. These problems can be solved by selecting the right type of PLLA by primary molecular weight, crystallinity, and residual monomers and by considering fabrication method.

Matsusue et al.[6] implanted rods made of PLLA in the femur bone marrow of rabbits and found no toxicity from drawn PLLA. Thin layers of fibrous tissue were formed at an early stage after implantation. The rods were completely absorbed after implantation, which was accompanied by the formation of thick, bony structures. However, it is not known whether the burr holes were completely replaced by bone. Böstman et al.[16] showed that most PGA was replaced with loose connective tissue. In clinical reports, X-rays often show an osteolytic area around PGA implants[17] and in rare cases, around PLLA implants.[5] A composite of bioactive hydroxyapatite (HA) and PLLA was created to solve these problems, and it successfully showed direct bone-bonding capability. However, the composites did not possess sufficient strength to be used clinically as bone-fixation devices.[18,19] Bone-fixation devices made of unsintered-HA (u-HA)/PLLA composites have higher strength and osteoconductivity and have shown satisfactory results in clinical use.[20–22]

The effect of exposure to resorbable material on nerve tissue in the epidural space after spinal surgery has not been reported. The purpose of this study was to compare u-HA/PLLA with PLLA in their histological effects, especially in terms of biocompatibility, on the spinal cord and nerve root in rabbits. We also sought to clarify their degradation behavior in the epidural space and to evaluate the effects of differing bioactivity and osteoconductivity in u-HA and calcined-HA (c-HA) composites with PLLA.

MATERIALS AND METHODS

Preparation of materials

The resorbable materials used in this study were uniform composite films composed of PLLA[23] and u-HA or c-HA, which are bioactive bioceramics. u-HA was synthesized by hydrolysis of pure aqueous calcium hydrogen phosphate and calcium carbonate at 90 °C. It was matured for 5 h, filtered, and fully dried for 10 h. It comprised microhexagonal prismatic crystals with an aspect ratio at 4–10 (2–3 × 0.3–0.5 μm) aggregated into clusters 5–50 μm across. These were milled and sieved, with the particle size limited to a range of 0.3–20 μm (average of ~3 μm). The Ca:P molar ratio by chemical analysis was 1.69, which is very close to that of pure hydroxyapatite (1.67). Fourier-transform infrared absorption spectra also identified this u-HA to be apatite containing carbonate, and X-ray diffraction identified particles with medium crystallinity. A 20 wt% u-HA aqueous suspension had pH 7.4–7.5 c-HA was made from u-HA by granulating and calcining at 900 °C to decarbonate it. The granules were crushed, milled, and sieved to limit their particle size to a distribution range and average similar to those of the u-HA particles. The molar ratio was 1.67, indicating that the c-HA was pure hydroxyapatite. It has already been demonstrated that u-HA is highly bioactive and totally absorbable, whereas c-HA is not totally absorbable but has a bioactive surface.[24]

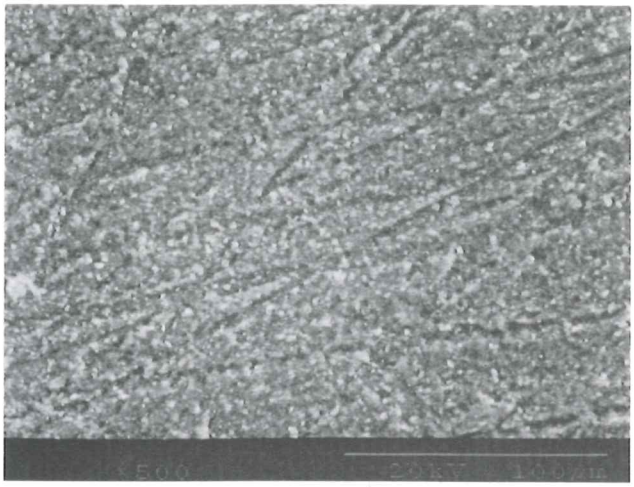
Films were fabricated by heat-compression flat plates prepared by casting u-HA/PLLA or c-HA/PLLA/dichloromethane solution. Films were 5 × 5 mm and 200-μm thick. The corners were planed off to avoid physical stimulus. Comparisons were made between u-HA/PLLA, c-HA/PLLA, and PLLA. We chose a PLLA with lower viscosity average molecular weight ($\overline{M_v}$) than the highest strength available for such composite materials (M_v about 22×10^4) to provide early degradation. The content of HA was 40 wt%. The surface of the u-HA/PLLA and c-HA/PLLA films was sanded so that HA particles were exposed, which was confirmed with scanning electron microscopy (SEM; Fig. 1).

Animal procedures

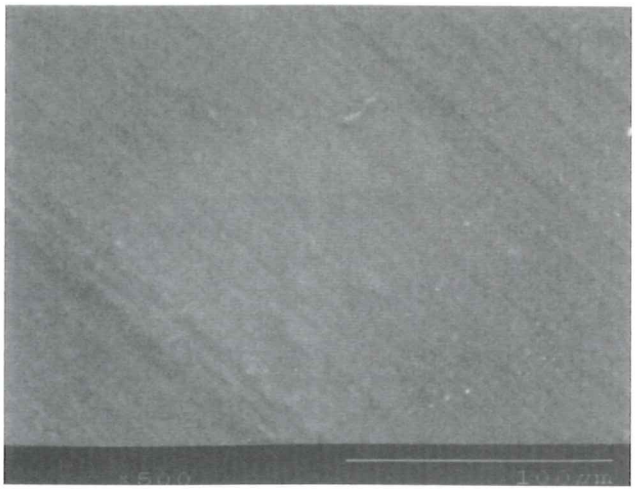
Devices were tested on 50 Japanese white rabbits weighing 3.0–3.8 kg. All the rabbits used in this experiment were housed in the animal center of Miyazaki Medical College. Pentobarbital 0.5 mL/kg was administered intravenously to the rabbits before surgery. They were sacrificed with excess pentobarbital after surgery. The following experimental manipulations were approved by the Experimental Animal Committee of Miyazaki Medical College.

Operation 1

Twenty rabbits were used for histological evaluation of the spinal cord, nerve root, and epidural space. A longitudinal midline incision was made on the back of the rabbits, and the lumbar laminae were exposed from L_4 to S_1. For partial laminectomy, a high-speed air drill with diamond burr was used. Under a magnifier, three intervertebral spaces and the epidural space were exposed. A portion of partial laminectomy region, the same size as the film was removed at the bifurcation of nerve root. u-HA/PLLA and PLLA films were implanted into the epidural space at each intervertebral space, while one space served as a control. The incision was cleaned with sterile saline and closed. The absence of paralysis in the extremities was confirmed after awaking from anesthesia. Histological analysis was per-



(A)



(B)

Figure 1. SEM findings of the surface of u-HA/PLLA composite (A) and PLLA (B).

formed in five rabbits at 4, 12, 25, and 50 weeks after the surgery. Microdissection was performed on the lumbar vertebrae using an air drill. The spinal cord and nerve root were then removed along with tissue in the epidural space and scar tissue. The films were removed together with the surrounding tissue. Histological evaluation was performed on each item.

Operation 2

The lumbar laminae were exposed in the same manner as in operation 1 and total laminectomy was performed at L_4 and L_5 levels in 30 rabbits to expose the epidural space. Two sheets each of u-HA/PLLA, c-HA/PLLA, and PLLA films were inserted into the epidural spaces and five sheets each of u-HA/PLLA and c-HA/PLLA films were implanted into the subcutaneous space. Epidural and subcutaneous films were removed from six rabbits at 4, 8, 12, 25, and 50 weeks after the surgery. $\overline{M_v}$ was measured and the surface of the film was observed with SEM and energy-dispersive X-ray (EDX) analysis. Degradation behavior and osteoconductivity on film surfaces was evaluated.

Histological evaluation

The nerve and surrounding tissue were fixed with neutral buffered 10% formalin and embedded in paraffin. Spinal cord and epidural tissue were cut into transverse serial sections, and the nerve root was cut into longitudinal and transverse serial sections. All specimens were stained with hematoxylin and eosin (HE) and observed by optical microscopy. Staining with Luxol Fast Blue and Bodian's methods and GFAP (glial fibrillary acidic protein) immunostaining were also performed for the sections from the spinal cord and nerve roots. Immunohistochemistry was performed on paraffin sections using an ordinary biotin-streptavidin method. Monoclonal antibodies used include those against GFAP (DAKO, Glostrup, Denmark), vimentin (DAKO), smooth muscle actin (DAKO), CD34 (DAKO), CD68 (DAKO), and desmin (DAKO). Nondecalcified hard tissue specimens around the films was fixed with 70% alcohol, dehydrated immediately after removal, and then embedded in methylmethacrylate resin. The blocks with embedded sections were sliced to 200-μm thickness with a hard-tissue-specimen slicer, ground to ~20-μm thickness with a hard-tissue grinder, and observed after Cole's HE staining.

Molecular weight measurement

After removing HA particles by filtering with a composite/chloroform solution, the $\overline{M_v}$ of the PLLA was calculated from the Mark-Houwik's formula[25] by measuring the intrinsic viscosity [η] at 25 °C using an Ostwald viscometer and substituting this value into the formula

$$[\eta] = 5.45 \times 10^{-4} \overline{M_v}^{0.73}$$

Student's *t*-test was used for statistical analysis of the samples.

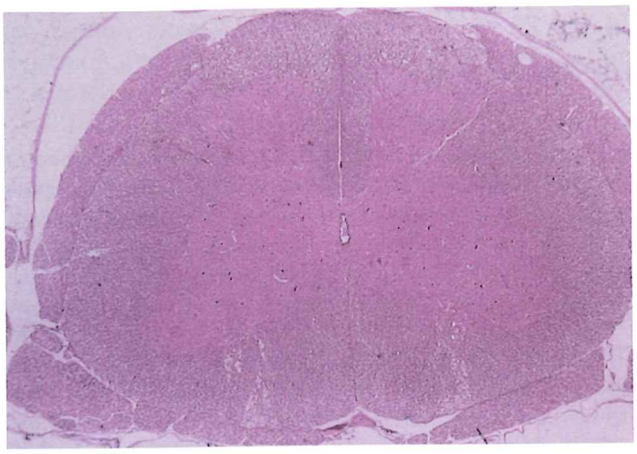
Bioactivity

The stoichiometric Ca/P molar ratio of deposited crystals was analyzed by SEM and EDX analysis.

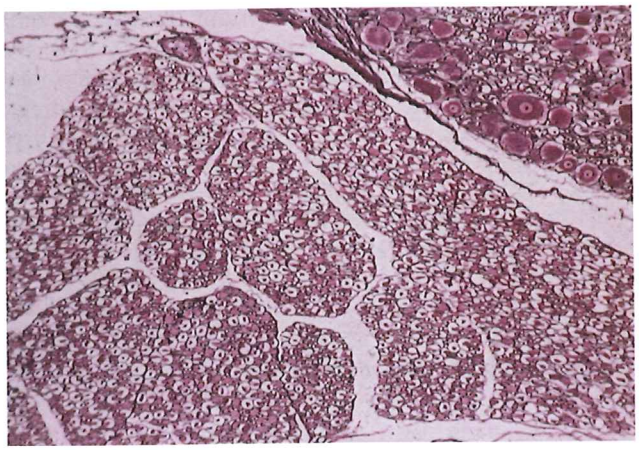
RESULTS

Postoperative course

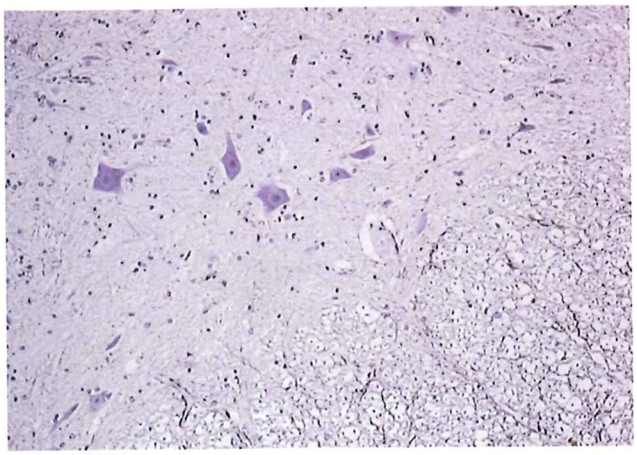
Three rabbits from operation 2 group were excluded due to the postoperative paralysis of the lower ex-



(A)



(B)



(C)

Figure 2. Photomicrographs of the spinal cord and the nerve root 50 weeks after implantation of the u-HA/PLLA, indicating no morphologic changes. (A) Cross section of the spinal cord (H&E stain, ×10). (B) Cross section of the nerve root (Bodian stain, ×50). (C) Cross section of the spinal cord (GFAP immunostaining, ×40).

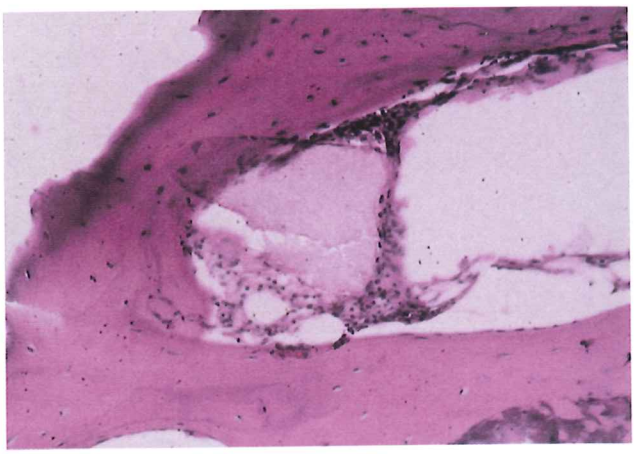
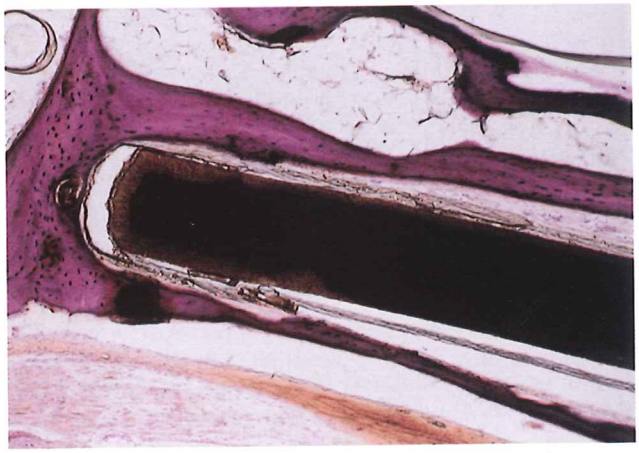
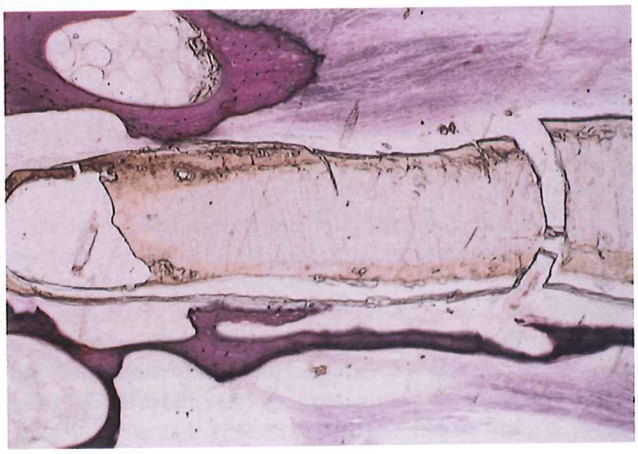


Figure 3. Photomicrographs of the u-HA/PLLA 50 weeks after implantation. Multinucleate giant cells engulfing the degradable polymer are found next to the bone (H&E stain, ×100).



(A)



(B)

Figure 4. (A) Photomicrograph of the u-HA/PLLA film 25 weeks after implantation. The implant is completely encased by reactive bone (H&E stain, ×40). (B) Photomicrograph of the PLLA film 50 weeks after implantation (H&E stain, ×40).

tremities. No paralysis was observed in the remaining rabbits. No infection or inflammation was seen at the incision site.

Histological study

Myelin loss, axonal damage, and gliosis were examined in the spinal cord and nerve root sections of 20 rabbits: 19 were found to be free of any abnormality (Fig. 2). One specimen in the spinal PLLA group showed a loss of anterior horn cells and mild gliosis in the anterolateral column after 25 weeks, probably due to the surgery; however, no lower extremity signs were observed.

Epidural tissue of the u-HA/PLLA and PLLA groups showed no significant histological differences. Collagenous connective tissue was edematous with exudation 4 weeks after the operation. Lymphocyte and plasma cell infiltration and hemosiderosis were seen, which was considered to be an inflammatory reaction due to surgery. No inflammatory cells were seen after 12 weeks. Reactive fibrosis was observed after 12 weeks. The fibroblastic cells were positive for vimentin and negative for smooth muscle actin, desmin, and CD34. Collagen fibers became dense after 25 weeks, and partial cartilaginous metaplasia and hyalinization were also observed.

The films were encapsulated by collagenous tissue, including fibroblasts, up to 25 weeks. Collagenous tissue was thicker at the back due to greater stress. The collagenous capsule was thinner at 50 weeks than at 25 weeks. The films were surrounded by macrophages inside the capsule in one specimen from the u-HA/PLLA group and two from the PLLA group. The films were also fragmented, and multinucleate giant cells of the foreign-body type were detected (Fig. 3). Uptake of film fragments into cells was observed with a polarizing microscope. New bone formation was seen along u-HA/PLLA films 4 weeks after surgery. Bone formation increased with time, and u-HA/PLLA films were encased by new bone 25 weeks after surgery. Osteoconductivity was higher in u-HA/PLLA than PLLA (Fig. 4). Collagenous tissue around the u-HA/PLLA films decreased along with an increase in new bone formation compared to the PLLA group. However, little direct bonding between bone and films occurred, as the films had not been stabilized.

$\overline{M_v}$ measurements

Baseline $\overline{M_v}$ values of u-HA/PLLA, c-HA/PLLA, and PLLA were 11.1×10^4, 9.91×10^4, and 8.71×10^4, respectively. $\overline{M_v}$ of u-HA/PLLA and c-HA/PLLA in epidural space decreased rapidly to 60–70% of the initial weight after 4 weeks. However, the rate of decrease was more gradual after that, reaching approximately 20% of the initial weight after 50 weeks. The $\overline{M_v}$ of PLLA decreased to ~80% of the initial weight after 4 weeks and to ~60% after 8 weeks, indicating that primary degradation was slower in PLLA compared with u-HA/PLLA ($p < 0.05$) and c-HA/PLLA. It decreased more slowly from 8 weeks than u-HA/PLLA and c-HA/PLLA ($p < 0.02$), but reached the same level as the other two groups after 50 weeks. There was no significant difference in the rate of $\overline{M_v}$ change between u-HA/PLLA and c-HA/PLLA (Fig. 5). The $\overline{M_v}$ of subcutaneous u-HA/PLLA was lower than that of c-HA/PLLA after 8 ($p < 0.005$) and 12 weeks ($p < 0.05$). However, there was no significance difference between the two groups from 25 weeks (Fig. 6). The pattern of $\overline{M_v}$ change for u-HA/PLLA and c-HA/PLLA was not significantly different between subcutaneous and epidural space. However, c-HA/PLLA implanted in the subcutaneous space

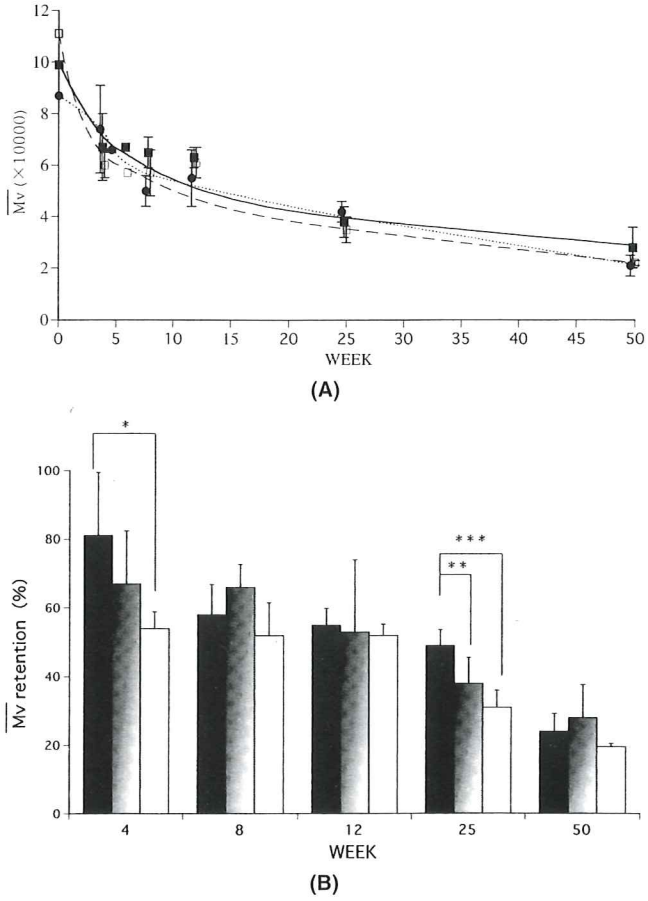


Figure 5. (A) Change in viscosity average molecular weight ($\overline{M_v}$) of HA/PLLA composites and PLLA with time in epidural space. ●, PLLA; ■, c-HA/PLLA; □, u-HA/PLLA. (B) Change in viscosity average molecular weight ($\overline{M_v}$) retention of HA/PLLA composites and PLLA with time in epidural space (*$p < 0.05$; **$p < 0.02$; ***$p < 0.005$). ■, PLLA; ■, c-HA/PLLA; □, u-HA/PLLA.

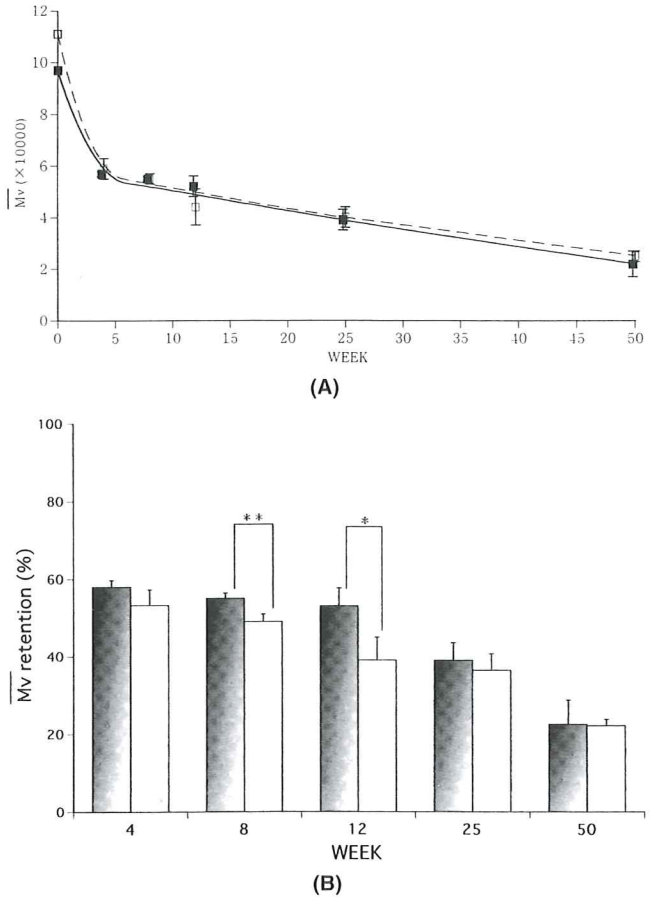


Figure 6. (A) Change in viscosity average molecular weight ($\overline{M_v}$) of HA/PLLA composites in subcutis. ■, c-HA/PLLA; □, u-HA/PLLA. (B) Change in viscosity average molecular weight ($\overline{M_v}$) retention of HA/PLLA composites with time in subcutis (*$p < 0.05$; **$p < 0.005$). ■, c-HA/PLLA; □, u-HA/PLLA.

showed a lower $\overline{M_v}$ than that in the epidural space after 8 weeks, and u-HA/PLLA from subcutaneous space showed a lower $\overline{M_v}$ than that in the epidural space after 12 weeks ($p < 0.02$). This may have been due to physical stress from the skin and reduced strength from the lower $\overline{M_v}$.

SEM and EDX analysis

There were no macroscopic changes on the surfaces of u-HA/PLLA or c-HA/PLLA. SEM showed that HA particles disappeared gradually with time. The surface of the PLLA film whitened after 50 weeks, probably because of an increase in crystallinity, but no conformational changes were observed with SEM. The surfaces of both u-HA/PLLA and c-HA/PLLA were covered with hard tissue that had grown from the epidural space from week 4, whereas soft tissue was seen on the surface of PLLA. There was no significant difference in the structure of hard tissue between u-HA/PLLA and c-HA/PLLA. Significant peaks of Ca and P were seen in hard tissue by EDX. THe Ca/P ratio was 1.45 to 1.55, which indicates that the hard tissue may be apatite deposited from the body (Fig. 7).

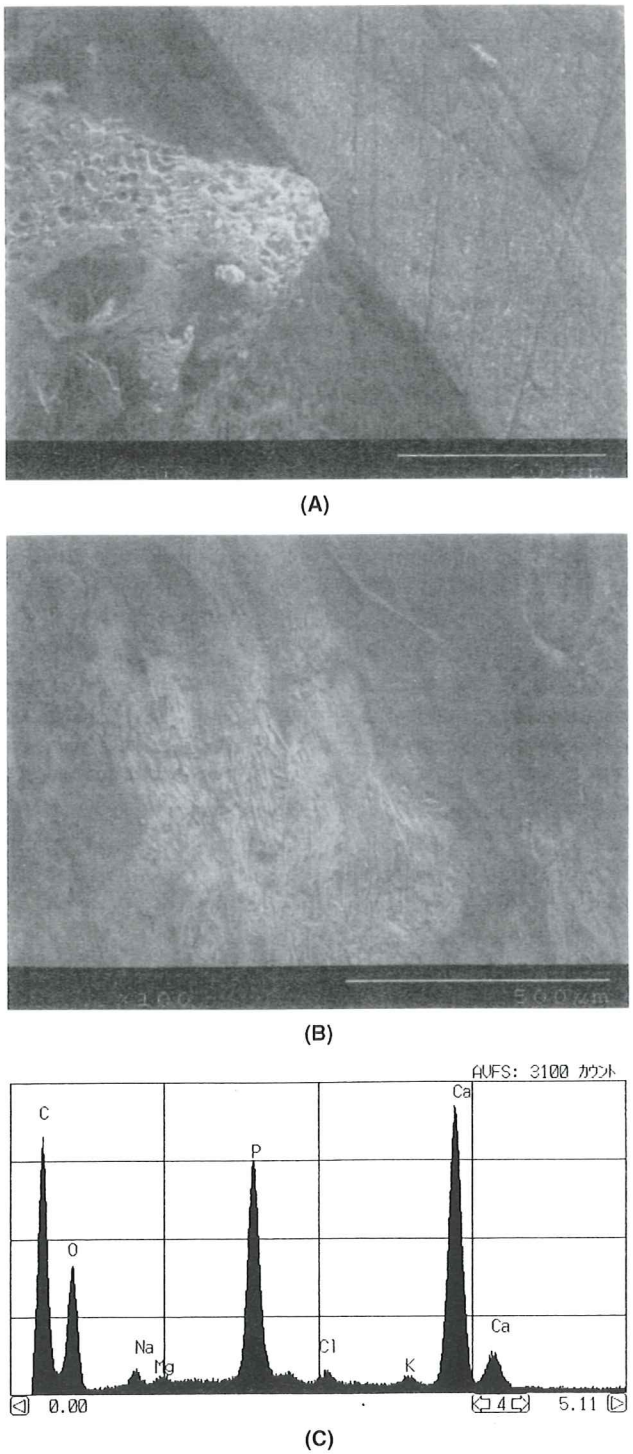


Figure 7. Change in SEM and EDX at the surface of a composite with time in epidural space. (A) c-HA/PLLA, 4 w; (B) u-HA/PLLA, 12 w; and (C) 4 w (EDX).

DISCUSSION

This study demonstrates the safety of HA/PLLA toward nerve tissue and the epidural space in spinal surgery. den Dunnen et al.[26–28] used PLLA/poly-ϵ-caprolactone as a guide to cross the sciatic nerve gap in rats and reported its effectiveness for regeneration and maturation of damaged nerve. Lundborg et al.[29] and Merle et al.[30] performed a clinical study of a silicon-polymer nerve guide; it had to be removed because of irritation. Satisfactory results of biocompatible, bioresorbable devices applied as guides to peripheral nerves,[31–33] especially PLLA,[34,35] have been reported.

This study also reports the effects of the new composite films on the spinal cord and nerve root in spinal surgery. The spinal cord has barriers such as dura, the subarachnoid membrane, and cerebrospinal fluid that are absent in peripheral nerves. However, there is no perineurium on nerve roots and the epineurium is so tenuous that it can be easily damaged by mechanical and chemical stimuli.[36,37] Our results show a good biocompatibility because there was no histological change in the nerve roots. Although we could show no visible abnormalities with Bodian and LFB, subtle histological changes in the nerve root would only be appreciable on plastic embedded sections and could be missed with our methods. However, good postoperative course without paralysis and inflammation may support our findings. Scar tissue that disturbs natural healing did not grow in the epidural space. It is therefore suggested that films implanted at the laminectomy site could have prevented fibrous tissue from breaking into the space. The pH of u-HA/PLLA composite suspension is neutral,[20] with no chemical-stimulating effect, and the biocompatibility of u-HA/PLLA inhibits cellular infiltration,[24,38] which might have contributed to the results.

No infiltration of inflammatory cells or foreign-body type giant cells was observed around PLLA or u-HA/PLLA films up to 25 weeks. However, phagocytosis by foreign-body giant cells was seen at 50 weeks. Previous studies have shown no inflammatory foreign body-reaction to PLLA,[39,40] whereas others have described infrequent reactions compared to PGA.[41,42] Factors that stimulate foreign-body reaction are believed to include gross size, quantity of per unit volume of tissue, shape, surface topography, and hydrophobicity/hydrophilicity of the particle surface. Significant fragmentation of the film, with advanced biodegradation, was observed at 50 weeks and was probably due to motion or stress on the film in the intervertebral joint. These factors may have caused foreign-body reactions.[43,44] One explanation to consider is that degradation of u-HA increases surface area and the number of pores, which accelerates further degradation. However, the tissue reactions of u-HA/PLLA were always less severe than those of PLLA. Because tissue reaction decreases as fragments are broken down to debris with lower molecular weight and because devices with high osteoconductivity are bonded to, and replaced by, surrounding bone, we can expect severe inflammatory foreign-body reactions to cease after about 6 months, following which synostosis becomes stable.

Biocompatibility and osteoconductivity of the films were confirmed even in the unstable condition where films were simply placed in the laminectomy site. Rapid degradation followed by a decrease in mechanical strength as seen with PGA tended to produce polymer debris at a rate far beyond the phagocytic capability of macrophages and to stimulate cells. On the other hand, the decrease in molecular weight of u-HA/PLLA and c-HA/PLLA was gradual, with rates being similar in both epidural and subcutaneous spaces. These results are similar to *in vitro* studies,[20] indicating the possibility that placement of the film in the spine causes little physical stress and diffusion of fluids. It is also possible that there was little enzymatic effect in the final phase of hydrolysis.[45,46] However, Hollinger[47] showed that the location of a polymer implant affects its degradation. The device should be designed to optimize the persistence of full prosthetic strength.

The $\overline{M_v}$ of u-HA/PLLA and c-HA/PLLA was significantly lower than that of PLLA, indicating that primary degradation was faster. This suggests that fluid immediately permeated through the interface between HA particles and PLLA and acquired a chance to hydrolyze. However, no significant decrease in mechanical strength resulted. This may have occurred because strength can be maintained until $\overline{M_v}$ drops to around 5×10^4. An increase in crystallinity may also have contributed.[20] The $\overline{M_v}$ of u-HA/PLLA may have decreased faster than that of c-HA/PLLA in subcutaneous space because u-HA/PLLA became more porous and the exposed surface increased after resorbable u-HA was released; this may have accelerated hydrolysis. Further investigation is therefore needed to elucidate the behavior of c-HA.

This study indicated that u-HA/PLLA and c-HA/PLLA had high osteoconductivity because of the bioactive and osteoconductive characteristics of HA particles. It is also worth considering that HA particles exposed on the surface of the device caused HA deposition in the body from an early stage and satisfactory new bone formation with time, as indicated by *in vitro* studies.[20] Rozema et al.[48] used PLLA for the treatment of a defective site in the orbital floor in goats and observed satisfactory bone coating, which was not seen in the control group. Our results showed early and long-lasting bone formation due to osteoconductivity in the u-HA/PLLA group, in addition to better bone coating of PLLA than in the control group. This

may have dispersed and reduced the biomechanical stress on the device and prevented fibrous tissue from interposing between the bone and surface of the device.[49] There was no significant difference in $\overline{M}_v$ decrease or the degradation process between u-HA and c-HA in epidural and subcutaneous spaces in this study. Further investigations should examine u-HA/PLLA composites for application to spinal surgery by focusing on the u-HA characteristics of high absorbency, bioactivity,[24,38] and biocompatibility, because it has the same Ca/P ratio as the body and includes carbonate.

In conclusion, bioresorbable u-HA/PLLA composite can be applied to spinal surgery because of the lack of any damages to the spinal cord and nerve roots. Its high osteoconductivity, even in the unstable conditions, at the laminectomy site indicates good prospects for use in spinal surgery.

The authors thank A. Ohno, Department of Pathology, Miyazaki Medical College Hospital, for his kind help in processing histological sections and immunohistochemistry.

References

1. Bullough PG. Metallosis. J Bone Joint Surg 1994;76-B:687–688.
2. Hatch RS, Strum PF. Late complication after single-rod instrumentation. Spine 1998;23:1503–1505.
3. Glassman SD, Johnson JR, Raque G. Management of iatrogenic spinal stenosis complication placement of a fusion cage. Spine 1996;21:2383–2386.
4. Matsusue Y, Yamamuro T, Oka M, Shikinami Y, Hyon SH, Ikada Y. *In vitro* and *in vivo* studies on bioabsorbable ultra-high-strength poly(L-lactide) rods. J Biomed Mater Res 1992; 26:1553–1567.
5. Yamamuro T, Matsusue Y, Uchida A, Shimada K, Shimozaki E, Kitaoka K. Bioabsorbable osteosynthetic implants of ultra high strength poly-L-lactide. A clinical study. Int Orthop 1994;18: 332–340.
6. Matsusue Y, Hanafusa S, Yamamuro T, Shikinami Y, Ikada Y. Tissue reaction of bioabsorbable ultra-high-strength poly(L-lactide) rods: a long term study in rabbits. Clin Orthop Rel Res 1995;317:246–253.
7. Tschakaloff A, Losken HW, Lalikos J. Experimental studies of DL-polylactic acid biodegradable plates and screws in rabbits: computed tomography and molecular weight loss. J Craniofac Surg 1993;4:223–227.
8. Viljanen J, Kimmunen J, Bondestam S, Majola A, Rokkanen P, Tormala P. Bone changes after experimental osteotomies fixed with absorbable self-reinforced poly-L-lactide screws or metallic screws studied by plain radiographs, quantitative computed tomography and magnetic resonance imaging. Biomaterials 1995;16:1353–1358.
9. Deguchi M, Boyle CC, Sato K, Matsuyama Y, Thomas AZ. Biomechanical evaluation of translaminar facet joint fixation. A comparative study of poly-L-lactide pins, screws, and pedicle fixation. Spine 1998;23:1307–1313.
10. Santavirta S, Konttinen Y, Saito T, Gronblad M, Partio E, Kemppinen P, Rokkanen P. Immune response to polyglycolic acid implant. J Bone Joint Surg 1990;72-B:597–600.
11. Böstman O, Hirvensalo E, Mäkinen J, Rokkanen P. Foreign-body reactions to fracture fixation implants of biodegradable synthetic polymers. J Bone Joint Surg 1990;72-B:592–596.
12. Böstman O, Partio E, Hirvensalo E, Rokkanen P. Foreign-body reactions to polyglycolide screws in 24/216 malleolar fracture cases. Acta Orthop Scand 1992;63(2):173–176.
13. Böstman O, Päivärinta U, Manninen M, Rokkanen P. Polymetric debris from absorbable polyglycolide screws and pins. Intraosseous migration studied in rabbits. Acta Orthop Scand 1992;63(5):555–559.
14. Bergsma JE, Rozema FR, Bos RR, de Bruijin WC. Foreign body reactions to resorbable poly(L-lactide) bone plates and screws used for the fixation of unstable zygomatic fractures. J Oral Maxillofac Surg 1993;51:666–670.
15. Bergsma JE, de Bruijin WC, Rozema FR, Bos RRM, Boering G. Late degradation tissue response to poly(L-lactide) bone plates and screws. Biomaterials 1995;16:25–31.
16. Böstman O, Päivärinta U, Partio E, Vasenius J, Manninen M, Rokkanen P. Degradation and tissue replacement of an absorbable polyglycolide screw in the fixation of rabbit femoral osteotomies. J Bone Joint Surg 1992;74-A:1021–1031.
17. Böstman OM. Osteolytic changes accompanying degradation of absorbable fracture fixation implants. J Bone Joint Surg 1991;73-B:679–682.
18. Verheyen CCPM, de Wijin JR, van Blitterswijk CA, de Groot K. Evaluation of hydroxyapatite/poly(L-lactide)composites: mechanical behavior. J Biomed Mater Res 1992;26:1277–1296.
19. Verheyen CCPM, de Wijin JR, Van Blitterswijk CA, de Groot K, Rozing PM. Hydroxyapatite/poly(L-lactide) composites: an animal study on push-out strengths and interface histology. J Biomed Mater Res 1993;27:433–444.
20. Shikinami Y, Okuno M. Bioresorbable devices made of forged composites of hydroxyapatite (HA) particles/poly-L-lactide (PLLA); part I. Basic characteristics. Biomaterials 1998;20:859–877.
21. Yasunaga T, Matsusue Y, Furukawa T, Shikinami Y, Okuno M, Nakamura T. Bonding behavior of ultra-high-strength unsintered hydroxyapatite particles/poly(L-lactide) composites to the surface of the tibial cortex in rabbits. J Biomed Mater Res 1999;47:412–419.
22. Furukawa T, Matsusue Y, Yasunaga T, Kobayashi M, Fujita H, Shikinami Y, Okuno M, Nakamura T. Ultra-high-strength hydroxyapatite particles/poly(L-lactide) composite rods for internal fixation of bone fractures; in vivo study. Bioceramics 1998; 11:419–422.
23. Hyon SH, Jamshidi K, Ikada Y. Polymer as biomaterials. New York: Plenum Press; 1984. p. 51–65.
24. Oonishi H, Hench LL, Wilson J, Sugihara F, Tsuji E, Kushitani S, Iwaki H. Comparative bone growth behavior in granules of bioceramic materials of various sizes. J Biomed Mater Res 1999; 33:31–43.
25. Schindler A, Harper D. Polylactide. II. Viscosity-molecular weight relationships and unperturbed chain dimensions. J Polym Sci 1979;36:360–380.
26. denn Dunnen WFA, Van der Lei B, Schakenraad JM, Blaauw EH, Stokroos I, Pennings AJ, Robinson PH. Long-term evaluation of nerve regeneration in a biodegradable nerve guide. Microsurgery 1993;14:508–515.
27. denn Dunnen WFA, Schakenraad JM, Zondervan GL, Pennings AJ, Van der Lei B, Robinson PH. A new PLLA/PCL copolymer for nerve regeneration. Mater Med 1993;4:521–525.
28. den Dunnen WF, Meek MF. Sensory nerve function and automutilation after reconstruction of various gap lengths with nerve guides and autologous nerve grafts. Biomaterials 2001;22:1171–1176.
29. Lundborg G, Dahlin LB, Danielsen N. Ulnar nerve repair by the silicon chamber technique. Scand J Plast Reconstr Hand Surg 1991;25:79–81.
30. Merle M, Dellon AL, Campbell JN, Chang PS. Complications

from silicon polymer intubation of nerves. Microsurgery 1989; 10:130–133.
31. Dellon AL, Mackinnon SE. An alternative to the classical nerve graft for the management of the short nerve gap. Plast Reconstr Surg 1988;82:849–856.
32. Mackinnon SE, Dellon AL. Clinical nerve reconstruction with a bioabsorbable polyglycolic acid tube. Plast Reconstr Surg 1990; 85:419–424.
33. Da Silva C, Madison R, Dikke SE, Chiu Th, Sidman R. An in vivo model to quantify motor and sensory peripheral nerve regeneration using bioresorbable nerve guide tubes. Brain Res 1985;342:307–312.
34. Evans GR, Brandt K, Widmer MS, Lu L, Meszlenyi RK, Gupta PK, Mikos AG, Hodges J, Williams J, Gurlek A, Nabawi A, Lohman R, Patrick CW Jr. In vivo evaluation of poly(L-lactic acid) porous conduits for peripheral nerve regeneration. Biomaterials 1999;20:1109–1115.
35. Widmer MS, Gupta PK, Lu L, Meszlency PK, Evans GR, Brandt K, Savel T, Gurlek A, Patrick CW Jr, Mikos AG. Manufacture of porous biodegradable polymer conduits by an extrusion process for guided tissue regeneration. Biomaterials 1998;19:1945–1955.
36. Yamagami T, Matsui H, Tsuji H, Ishimura K, Sano A. Effect of laminectomy and retained extradural foreign body on cauda eqiona adhesion. Spine 1993;18:1774–1781.
37. Chiba K. An experimental study on the pathological changes of the intervertebral disc and its surrounding tissues after intradiscal injection of various chemical substances (the first report). J Jpn Orthop Assoc 1993;67:1055–1069.
38. Sakae T, Kozawa Y, Akagi I, Inoue M, Nagatuka H, Nagai N, Matsuda N, Wakana Y, Kimura K, Suzuki M. Crystallographic properties and cell affinity study of a series of synthetic hydroxyapatites (in Japanese). Nihon Univ J Oral Sci 1994;20:40–48.
39. Pihlajamäki H, Bostman FR, Hirvensalo E, Törmälä P, Rokkanen P. Absorbable pins of self-reinforced poly-L-lactic acid for fixation of fractures and osteotomies. J Bone Joint Surg 1992;74-B:853–857.
40. Majola A, Vainionpää S, Vihtonen K, Mero M, Vasenius J, Tormala P, Rokkanen P. Absorption, biocompatibility, and fixation properties of polylactic acid in bone tissue: an experimental study in rats. Clin Orthop Rel Res 1991;268:260–269.
41. Bucholz RW, Henry S, Henley MB. Fixation with bioabsorbable screws for the treatment of fractures of the ankle. J Bone Joint Surg 1994;76-A:853–857.
42. Böstman O. Osteoarthritis of the ankle after foreign-body reaction to absorbable pins and screws: a three- to nine-year follow-up study. J Bone Joint Surg 1998;80-B:333–338.
43. Matlaga BF, Yasenchak LP, Salthouse TN. Tissue response to implanted polymers: The significance of shape. J Biomed Mater Res 1976;10:391–397.
44. Lam KH, Schakenraad JM, Nieuwenhuis P. The influence of hardness, wettability and fragmentation of polymers on the inflammatory response. In: Biocompatibility of degradable biomaterials. Thesis, Groningen, The Netherlands (ISBN 90-9005022-1, 1992).
45. Williams DF. Enzymic hydrolysis of polylactic acid. Eng Med 1981;10:5–7.
46. Schakenraad JM, Hardonk MJ, Feijen J, Molenaar I, Nieuwenhuis P. Enzymatic activity toward poly(L-lactic acid) implants. J Biomed Mater Res 1990;24:529–545.
47. Hollinger JO. Biodegradable bone repair materials. Synthetic polymers and ceramics. Clin Orthop 1986;207:290–305.
48. Rozema FR, Bos RRM, Boering G, Pennings AJ, Jansen HWB. Poly(L-lactide) implants in repair of defects of the orbital floor: an animal study. J Oral Maxillofac Surg 1990;48:1305–1309.
49. Sevastjanova NA, Mansurova LA, Dombrovska LE, Slutskii LI. Biochemical characterization of connective tissue reaction to synthetic polymer implants. Biomaterials 1987;8:242–247.